Scientific African 7 (2020) e00316

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Effects of three herb-based alcoholic beverages manufactured in Ghana on sperm characteristics and reproductive hormones in rats

Robert P. Biney[a,*], Phyllis E. Owusu Agyei[a], Elvis O. Ameyaw[b], John K. Afortude[b], Isaac T. Henneh[a], Ernest Obese[a], Samuel Badu Nyarko[c], Martins Ekor[a]

[a] Department of Pharmacology, University of Cape Coast, Cape Coast, Ghana
[b] Department of Biomedical Sciences, University of Cape Coast, Cape Coast, Ghana
[c] School of Medical Sciences, University of Cape Coast, Cape Coast, Ghana

ARTICLE INFO

Article history:
Received 5 November 2019
Revised 29 January 2020
Accepted 4 February 2020

Editor: Dr. B Gyampoh

Keywords:
Spermatotoxicity
Alcoholism
Herbal medicines
Herb-based alcoholic beverages
Male reproduction

ABSTRACT

Background: Advertisements of most alcoholic beverages in Ghana persuasively entice consumers with herbal constituents believed to enhance sexual performance, although, alcohol has negative effect onfertility. The impact of herbal constituents in these alcoholic beverages on the known negative effects of alcohol on fertility remains to be evaluated. This study evaluated the effects of three alcoholic beverages manufactured in Ghana on male reproductive function in rats.

Methods: Male Sprague-Dawley rats received 0.5, 2.5 or 5 ml/kg of 3 herb-based alcoholic beverages (Brand A, B & C), 42% $^{v}/_{v}$ ethanol or distilled water *p.o.* for 21 days. On day 22, blood was collected and assayed for serum prolactin, testosterone and, hematological parameters. Testes were removed for histology and sperm from the cauda epididymis analyzed for sperm count, motility and morphology. Total antioxidant capacity of the testes was determined.

Results: The three alcoholic beverages did not cause any changes in hematological parameters and relative weight of the testes. However, Brand C reduced epididymal sperm count and increased serum prolactin and induced loss of germinal epithelial cells, necrosis, intertubular edema in seminiferous tubules and lipid and fluid accumulation in the Sertoli cells. Both Brands A and B caused tubular atrophy and enlarged spermatogonia at 5 ml/kg. All three brands significantly ($p<0.001$) reduced sperm motility and serum testosterone, although they increased total antioxidant capacity.

Conclusion: The herb-based alcoholic beverages evaluated did not enhance or exert a beneficial effect on male reproductive function but rather demonstrated spermatotoxic properties irrespective of the presence of medicinal plants.

* Corresponding author.
E-mail addresses: robert.biney@ucc.edu.gh (R.P. Biney), p.owusuagyei@uccsms.edu.gh (P.E. Owusu Agyei), eameyaw@ucc.edu.gh (E.O. Ameyaw), i.t.henneh@uccsms.edu.gh (I.T. Henneh), e.obese@uccsms.edu.gh (E. Obese), s.badunyarko@uccsms.edu.gh (S. Badu Nyarko), m.ekor@uccsms.edu.gh (M. Ekor).

https://doi.org/10.1016/j.sciaf.2020.e00316

Introduction

Alcoholism is a primary, chronic relapsing disease of brain reward, motivation, memory, and related circuitry. It is marked by excessive and compulsive drinking of alcohol which progresses into physiological and physical dependence accompanied by clinically significant impairment or distress. [1] Alcohol has known toxic effects on the reproductive functions in both males and females. The hypothalamus, the anterior pituitary gland, and the gonads (HPG) axis can malfunction under the influence of alcohol, to cause impotence, infertility and reduced secondary sexual characteristics in males. [2,3] Other specialized cells involved in normal reproductive functioning are also negatively impacted by chronic alcohol consumption. The Leydig cells responsible for the production and secretion of testosterone are adversely affected by alcohol leading to a decrease in serum testosterone. [4] Additionally, the significant role of testicular Sertoli cells in sperm maturation is impaired under the influence of alcohol. Furthermore, hormone production and release from the anterior pituitary gland and hypothalamus can also be compromised by alcohol. [5] In rodents, an alcohol-rich diet induces anomalies in sperm parameters and alterations in the reproductive tract. [6]

Despite these known negative effects of alcohol on reproductive function, there are numerous reports of increased consumption of alcoholic beverages in most parts of the world. Alcoholic beverages are mostly consumed by both males and females in large quantities and alcoholism has become a major public health concern. In Ghana, 43% of the youth consume some form of alcohol and there is growing concern about the increase in alcohol consumption and its undesirable health outcomes. [7,8] This increased consumption of alcoholic beverages has been enhanced by the airing of enticing advertisements on various electronic media. [8] Osei-Bonsu et al., reports in a survey of Ghanaian youths that, one in five respondents cite advertisements as the main influence for patronizing alcoholic beverages. This newfound love for alcoholic beverages stems from the fact that these new alcoholic beverages are herb-based. They are thus touted as having medicinal properties not limited to good health and sexual vitality. Thus, it is very enticing to take in these herb-based alcoholic beverages (HBABs).

The impact of the herbal constituents in these alcoholic beverages on the known negative effects of alcohol on fertility remains to be evaluated even though several studies have been conducted on the effects of ethanol on male fertility. This study addresses this gap in knowledge by evaluating the effects of three popular herb-based alcoholic beverages manufactured in Ghana on serum sex hormone levels, sperm characteristics and testicular histology in male Sprague Dawley rats.

Materials and methods

Sampling of locally-manufactured herb-based alcoholic beverages

The three (3) most common locally produced brands of alcoholic beverages were sampled and purchased from the five drinking pubs in Cape Coast. Both the selection of drinking pubs and the subsequent sampling of the top three brands were by convenient sampling. [9] The 3 brands of beverages sampled were listed in the product information packs to contain *Xylopia aethiopica, Khaya senegalensis, Anthocleista nobilis* and *Zingiber officinale* as herbal constituents. All 3 samples had the same manufacturer-listed concentration of ethanol (42% $^{v}/_{v}$). They were randomly assigned groups as Brand A, B, and C. Brands A and B contained similar herbal constituents (*Xylopia aethiopica, Khaya senegalensis,* and *Anthocleista nobilis*) while Brand C contained *Zingiber officinale* as the sole herbal constituent.

Animals

Fifty-five healthy male Sprague-Dawley rats (130–200 g) were obtained from the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana. The experimental animals were housed and acclimatized for two weeks in the animal facility of the Department of Biomedical Science, University of Cape Coast (UCC), Cape Coast, Ghana, prior to the experiments. They were housed in stainless steel cages with softwood shaving as bedding, cleaned and maintained daily in an environmentally controlled room provided with 12/12 h light/ dark cycle and maintained at 25 °C. They were fed with standard commercial rodent pellets and water *ad libitum*. The mice were handled humanely throughout the experimental period. All experiments were in accordance with National Institutes of Health (NIH) guidelines for care and use of laboratory animals. Experimental protocols were subjected to scrutiny and approval by the Animal Ethics Committee of the University of Cape Coast Institutional Review Board (UCCIRB) under the approval identifier UCCIRB/CoHAS/2018/062.

Experimental design

The rats were randomly assigned to 5 main groups i.e. A, B, C, D and E. Groups A, B and C were assigned to Brands A, B, and C respectively. Subsequently, each group was subdivided into 3 subgroups to yield eg. A1, A2 and A3 $n = 5$ rats in each subgroup. Rats in group A1, A2, and A2 received 0.5, 2.5 or 5 *ml*/*kg* of Brand A. Rats in Group D ($n = 5$) received 2.5 *ml*/*kg* of 42% $^{v}/_{v}$ ethanol while rats in Group E ($n = 5$) received only 10 *ml*/*kg* distilled water as detailed in Table 1. Rats in their various groups received twice daily for 21 days, either Brands A, B, C, 42% $^{v}/_{v}$ ethanol or distilled water administered orally via an intragastric gavage. Doses were based on the reported amount consumed daily during the pilot study. The smallest

Table 1
Experimental treatment groups used in the study.

Treatment group		Dose
A	A1	0.5 ml/kg Brand A
	A2	2.5 ml/kg Brand A
	A3	5.0 ml/kg Brand A
B	B1	0.5 ml/kg Brand B
	B2	2.5 ml/kg Brand B
	B3	5.0 ml/kg Brand B
C	C1	0.5 ml/kg Brand C
	C2	2.5 ml/kg Brand C
	C3	5.0 ml/kg Brand C
D		2.5 ml/kg of 42% $^{v}/_{v}$ ethanol
E		10 ml/kg distilled water

dose was computed from the minimum reported dose of 1 shot/day (30 ml/day) with the middle dose being the average reported dose and highest dose being 10 times the minimum reported dose. The same dosing schedule was used for brands B and C (3 subgroups per brand $n = 5$ in each subgroup). Twenty-four hours after the last daily administration, the rats were euthanized to remove the testes for histological analysis while the cauda epididymis was removed for semen analysis. Blood was collected into gel separator tubes through cardiac puncture to determine serum concentrations of testosterone and prolactin.

Semen collection and analysis

The left caudal epididymis was separated from the testis, minced and placed in 6 *ml* of PBS (pH =6.8–7.4) pre-warmed at 35–37 °C. Sperm characteristics were evaluated with a slight modification of the procedure described. [10] A total of 10 µl sperm suspension was pipetted from the sperm suspensions and loaded unto an improved Neubauer haemocytometer. A 30 s video of 4 fields of view (magnification = × 10) on each slide was recorded with a camcorder (Olympus U-TV1Xc) fitted to the microscope (Olympus CX41, Tokyo, Japan). This was followed by sperm counting using procedures described by Luthfi. [11] Sperm count was calculated as the total number of sperms per 1 ml cauda epididymis. Sperm motility was assessed from the recorded videos by tracking the distance travelled by the sperm using the MTrackJ plugin of ImageJ software. Distance travelled by each sperm in µm was measured and average speed for 20 sperms in $\mu m/s$ from each slide were obtained. The removed testes were weighed and the left testes from each rat stored in 10 ml of 10% formalin for histology analyses. Four (4) μm sections were cut and stained with hematoxylin and eosin for routine histological analyses.

Determination of serum testosterone and prolactin

To measure serum testosterone and prolactin levels in treated and control animals, collected blood samples were centrifuged at 2500 *rpm* for 5 min. The serum obtained was collected and stored at −20 °C. Serum testosterone and prolactin concentrations were determined with enzyme-linked immunosorbent assay (ELISA) kits according to manufacturers' outlined protocols (testosterone: Rat Testosterone ELISA Kit EK7014, Boster Biological Technology, Pleasanton, CA, USA; prolactin: Prolactin PicoKine^{TM} ELISA Kit EK0594, Boster Biological Technology, Pleasanton, CA, USA). Absorbances were read with a Microplate reader (URIT-660 Analyzer, URIT Medical Electronics, Guangxi, China).

Statistical analysis

Data obtained is expressed as bar graphs of the mean ± S.E.M. All data were assessed for normality using the D'Agostino-Pearson test for normality. Data was subsequently analysed with a parametric test, analysis of variance. Where the difference between the means compared by one-way analysis of variance (ANOVA) was found to be significant, the specific groups in which significance is reported were further analaysed using Dunnet's test as *post hoc* test. In all statistical tests, $p < 0.05$ was considered significant. All analysis was performed using GraphPad Prism 7 software for windows (GraphPad Prism, San Diego, USA).

Results

Effect of selected brands of HBABs on testicular weight and sperm characteristics

Treatment with all brands of the sampled herb-based alcoholic beverages showed no significant change in the relative weight of testes as compared with the naïve group as shown in Fig. 1a. Administration of HBABs Brand A and B yielded no significant changes in sperm count compared to the naïve animals. However, Brand C-treated rats (0.5, 2.5, 5 ml/kg) showed significant ($p < 0.01$) reduction in the epididymal sperm count when compared to naïve animals as represented in Fig. 1b.

After 21 days of treatment with the various samples of HBABs, sperm motility significantly reduced compared to ethanol in 2.5 ml/kg and 5 ml/kg of Brand B ($p<0.01$) and all doses of Brand C ($p<0.01$) as observed is in Fig. 1c. There was also a

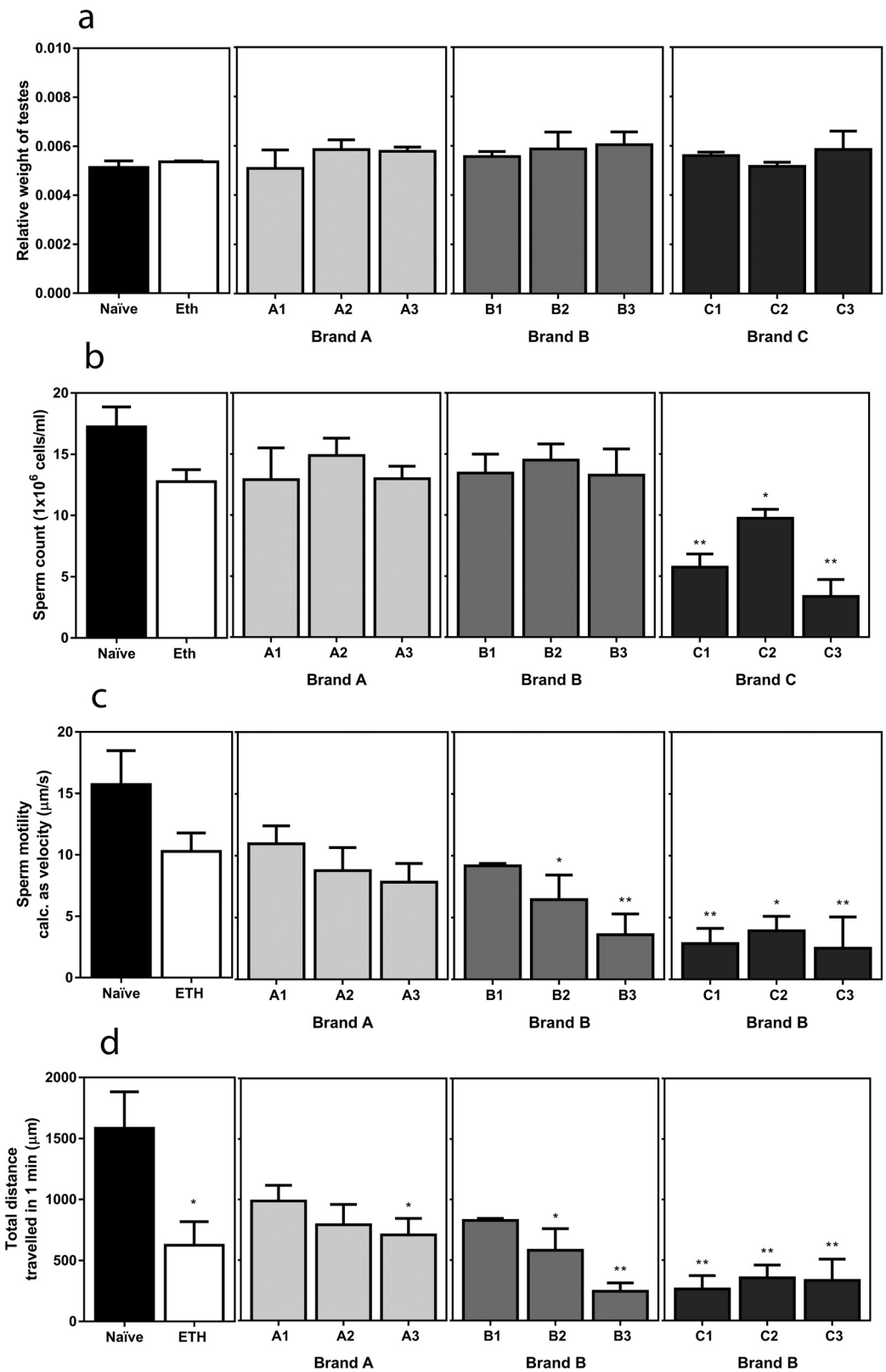

Fig. 1. Effect of various brands of herb-based alcoholic beverages (HBABs) and ethanol on rat testicular weight (a) sperm count (b) and sperm motility (c and d) after 21 days of treatment. ** $p < 0.01$ and * $p < 0.05$ compared to the naïve group.

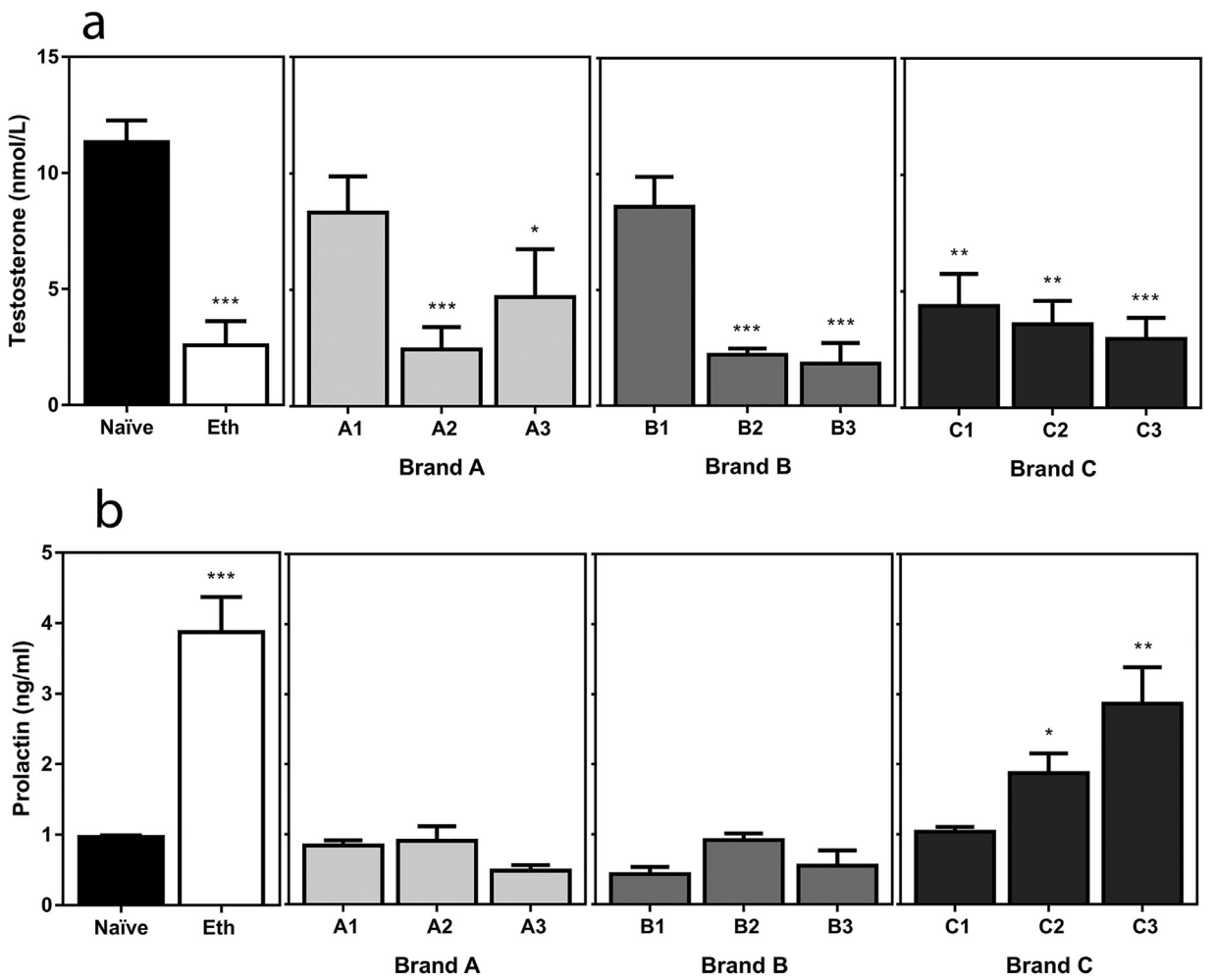

Fig. 2. Serum testosterone (a) and prolactin (b) concentrations of ethanol, brands A, B, and C-treated rats after 21 days of treatment. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ compared to the naïve group.

significant reduction in total distance traveled by sperms of rats that received 5 *ml*/*kg* of Brand A ($p = 0.0428$), 2.5 and 5 *ml*/*kg* of Brand B ($p<0.01$) and all doses of Brand C ($p<0.01$) compared to the naïve control just as was observed in ethanol treatment group Fig. 1d.

Effect on serum testosterone and prolactin

Serum testosterone levels in all groups were significantly reduced after treatment ($p < 0.001$). However, the small doses (0.5 *ml*/*kg*) in brand A and B showed no significant change as compared to the naïve group showed in Fig. 2a. Prolactin levels in ethanol and brand C-treated mice were significantly increased when compared to the naïve group after treatment ($p<0.01$). However, brands A and B showed no statistically significant changes in serum prolactin when compared to the naïve rats as shown in Fig. 2b.

Effects of HBABs on the histology of testes

The 21-day treatment with all the brands of HBAB resulted in increased luminal diameter of seminiferous tubules in all doses as compared with the naïve group. These results were similar to the ethanol treatment group. Additionally, thickening of the basal layer of epithelium tubules, tubular atrophy and intertubular edema characterized by eosinophilic infiltration in the interstitium was also observed in all the brands but most prominently in Brand C as shown in Fig. 3. Morphometric evaluation of spermatozoa of the treated rats revealed similar deleterious effects on rats that were treated with the HBABs when compared to the ethanol group. Furthermore, treatment with the HBABs resulted in enlarged spermatogonia in addition to lipid and fluid accumulation in the Sertoli cells. There was also disorientation of germinal epithelium and necrosis characterized by fading of the chromatin of germinal epithelial cells of the seminiferous tubules across all groups compared to the naïve group as shown in Fig. 3C top panel.

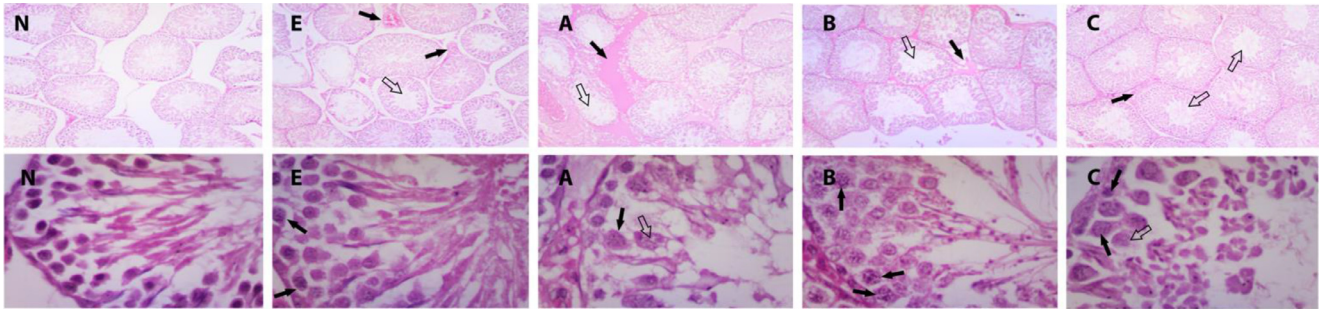

Fig. 3. Effects of various HBABs and ethanol on testicular histology
Top panel: Representative histological slides showing inter-tubular oedema—eosinophilic infiltration of the interstitium (hollow arrows) and dilation of seminiferous tubules (bold arrows) in the testes of naïve (N), ethanol (E), Brand A 5 *ml*/*kg* (A), Brand B 5 *ml*/*kg* (B) and Brand C (5 *ml*/*kg*)-treated rats after 21 days of treatment. Magnification = × 40. **Bottom panel:** Spermatogonia undergoing necrosis (bold arrows) and exhibiting vacuolation (hollow arrows) in naïve (N), ethanol (E), Brand A 5 *ml*/*kg* (A), Brand B 5 *ml*/*kg* (B) and Brand C (5 *ml*/*kg*)-treated rats after 21 days of treatment. Magnification = × 400.

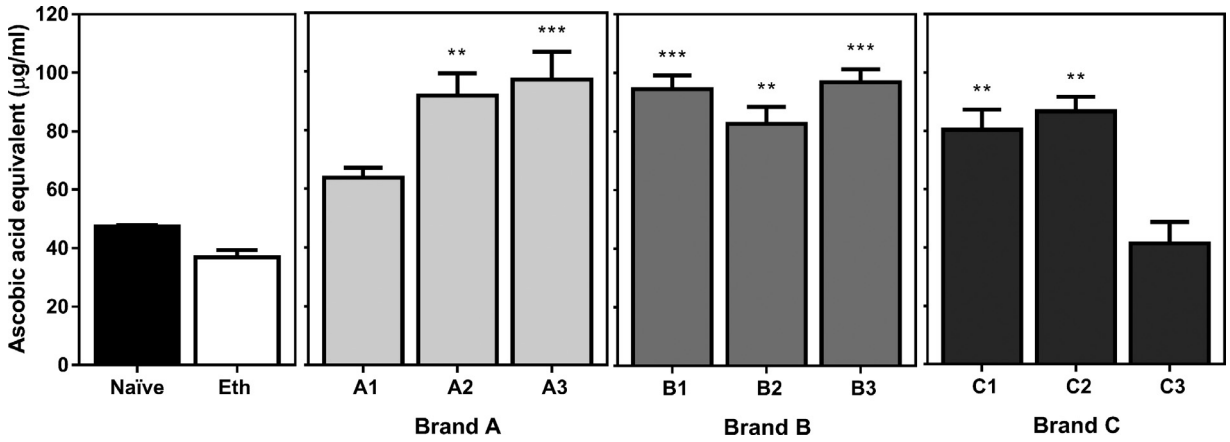

Fig. 4. Effect of ethanol, brands A, B and C on total antioxidant capacity of rat testes after 21 days of treatment. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ compared to the naïve group.

Effect on total antioxidant capacity of testes

With the exception of 0.5 *ml*/*kg* Brand A and 5 *ml*/*kg* Brand C, all other tested doses of Brands A, B and C exhibited significant antioxidant effects measured as equivalent antioxidant capacity of ascorbic acid in $\mu g/ml$ as represented in Fig. 4.

Discussion

Results from our data indicate that contrary to popular belief in Ghana, herb based-alcoholic beverages (HBABs) caused some deleterious effects to reproductive functions in male mammals; specifically, Sprague-Dawley rats. Despite the significant total antioxidant capacity of the samples evaluated, microscopic testicular deformities were observed in rats treated with all HBABs brands sampled and tested.

Ethanol is a known spermatotoxin. [6,12] There is a popular assumption that by combining it with medicinal plants, the deleterious effects of alcohol might be prevented by the medicinal actions of these plants. The samples evaluated (which were the most popular in the study area), contained either *Khaya senegalensis, Xylopia aethiopica, Anthocleista nobilis* or *Zingiber officinale* as the base herbs. Several medicinal properties have been attributed to these herbs. *Khaya senegalensis* possesses anti-inflammatory and anti-plasmodial effects [13] while *Xylopia aethiopica* has demonstrable anti-inflammatory [14], analgesic [15], and antidepressant [16] properties. *Anthocleista nobilis* has been reported to possess wound healing and anti-plasmodial effects. [17] *Zingiber officinale* has anti-inflammatory and anti-thrombosis effects. [18] All four medicinal herbs possess potent antioxidant properties and were therefore expected to mitigate some of the known deleterious effects of ethanol on the reproductive organs. [19,20,21]

However, earlier reports indicate that some of the herbs contained in the sampled HBABs in themselves have certain undesirable effects on male reproduction functioning. It has been shown that the extract of *K. senegalensis* causes a depletion of spermatogenic cells with a corresponding loss of architecture of seminiferous tubules which could result in male infertility. [22] It is thus not surprising that similar observations are seen in HBAB Brands A and B both of which contained *K. senegalensis.* Furthermore, Nwanga [23] and Woode et al. [10], have both showed independently similar changes in sperm count and functionafter the administration of ethanolic extracts of *Xylopia aethiopica* on the male reproductive organ in Wistar rats.

HBAB Brand C-treated rats exhibited the most profound reduction in sperm count in contrast to the ethanol group and Brands A and B-treated rats. Precipitous reduction in sperm count associated with no significant testicular weight change seen in the study could be attributed to a significant decrease in serum testosterone levels with concomitant destruction to Leydig cells and spermatogonia which was observed in Brand C treated rats. [3] Pressure buildup from the elevated volume of seminiferous tubule fluid within the lumen of tubules accounts for the thinning and compression of the seminiferous epithelium and the loss/degeneration of epithelial germ cells located in the tubules. These elevated volumes may arise due to impaired or decreased reabsorption of fluid in the efferent ducts, decreased emptying of fluid from the seminiferous tubules, or increased production of seminiferous tubule fluid by the Sertoli cells. This can then lead to seminiferous tubular dilation with a sequel of atrophy. [24] The presence of seminiferous dilation and intertubular edema in HBABs treated groups similar to the ethanol treatment group is an indication of the direct effect of HBABs on the testicular tissue irrespective of their herbal constituents.

The successful and complete male germ cell maturation and development is dependent on the balanced endocrine interplay of the hypothalamus and pituitary hormones and the testes. [5] Prolactin is inversely related to testosterone con-

centrations [25] and elevated prolactin in males suppresses serum testosterone level. [5] There was a significant reduction of serum testosterone occurring in all the HBABs tested with corresponding elevated levels of prolactin in Brand C. The reduction in testosterone levels could also be linked with the significant fluid accumulation observed in the Leydig cells and seminiferous tubules. This can result in the induction of necrosis in Leydig cells with a subsequent decrease in their number which may account for reduced production. [10]

In alcoholics, there is a suppression of prolactin response to thyrotropin-releasing factor suggesting a gonadotropin deficiency in these individuals. Gonadotropin-releasing hormone (GnRH) is known to stimulate luteinizing hormone (LH) production which in turn stimulates testosterone production and hence, low testosterone levels and elevated prolactin levels observed in this study could also be attributed to impaired GnRH production. It is thus possible that the medicinal herbs acted in synergy with ethanol to act as a direct insult to the Sertoli cells, testosterone-producing cells (Leydig cells) or anterior pituitary gland responsible for follicle-stimulating hormone (FSH) production to adversely affect sperm motility. Reduction in motility could also be associated with the immense mortality of Sertoli cells characterized by necrosis and vacuolation observed in the histological analyses.

Ethanol reduces the energy (ATP) required for the movement of the flagella of sperms by reducing mitochondria function and thus impeding sperm motility. [25] With the mitochondria as a primary site for the generation of free radicals, an inhibition of mitochondrial function leads to increased oxidative stress. [26] Despite all the HBABs tested having a high total antioxidant capacity, reduced sperm motility was observed in all the brands similar to the effects seen in ethanol alone. Thus, the findings of the present work indicate that all the 3 sampled HBABs may promote infertility by altering the functioning of the testes and distort sperm characteristics.

In conclusion, the results obtained from the present study suggest that all the alcoholic beverages caused a significant decrease in serum testosterone, sperm motility, and sperm count by causing disorganization and loss of the germinal epithelium of the seminiferous tubules of the testes irrespective of their herbal constituents. It is thus recommended that consumers of such products should be mindful of the potentially deleterious effects of locally manufactured herb-based alcoholic beverages.

Authors contribution

RPB, EOA, EO participated in the hypothesis conceptualization, experimental design, data collection and analysis, discussion and writing the manuscript paper. PEOA, JKA, ITH, and SBN contributed to the data collections and analysis manuscript witting. ME contributed to the hypothesis conception, experimental design and analysis, and discussion. All authors reviewed the manuscript.

Declaration of Competing Interest

The authors do not have any conflict of interest to declare.

Acknowledgments

The authors are grateful to Mr. Ernest Asiamah of the Department of Biomedical Sciences for his support with the histological analysis.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] F.T. Crews, R.P. Vetreno, Mechanisms of neuroimmune gene induction in alcoholism, Psychopharmacology (Berl.) 233 (9) (2016) 1543–1557, doi:10.1007/s00213-015-3906-1.
[2] R.A. Condorelli, A.E. Calogero, E. Vicari, S. La Vignera, Chronic consumption of alcohol and sperm parameters: our experience and the main evidence, Andrologia 47 (4) (2015) 368–379, doi:10.1111/and.12284.
[3] G.R. Rompala, A. Mounier, C.M. Wolfe, Q. Lin, I. Lefterov, G.E. Homanics, (Heavy chronic intermittent ethanol exposure alters small noncoding RNAs in mouse sperm and epididymosomes, Front. Genet. 9 (2018) 32, doi:10.3389/fgene.2018.00032.
[4] A.S. Vore, T. Doremus-Fitzwater, A. Gano, T. Deak, Adolescent ethanol exposure leads to stimulus-specific changes in cytokine reactivity and hypothalamic-pituitary-adrenal axis sensitivity in adulthood, Front. Behav. Neurosci. 11 (2017) 78, doi:10.3389/fnbeh.2017.00078.
[5] A. Sansone, C. Di Dato, C. de Angelis, D. Menafra, C. Pozza, R. Pivonello, …, D. Gianfrilli, Smoke, alcohol and drug addiction and male fertility, Reprod. Biol. Endocrinol. 16 (1) (2018) 3, doi:10.1186/s12958-018-0320-7.
[6] M. Price, F. Yang, J.H. Sisson, M. Wirschell, Ciliary dynein dysfunction caused by chronic alcohol exposure., in: S.M. King (Ed.), Dyneins, 2nd Edition, Academic Press, 2018, pp. 402–417, doi:10.1016/B978-0-12-809470-9.00016-3.
[7] E. Osei-Bonsu, P.K. Appiah, I.D. Norman, G.A. Asalu, M. Kweku, S.Y. Ahiabor, …, S. Boadu, Prevalence of alcohol consumption and factors influencing alcohol use among the youth in Tokorni-Hohoe, volta region of Ghana, Sci. J. Public Health 5 (3) (2017) 205–214, doi:10.11648/j.sjph.20170503.18.
[8] Ministry of Health of Ghana, Ghana national alcohol policy, 2017. Retrieved from http://www.moh.gov.gh/ghana-launches-national-alcohol-policy/ 21/01/2020
[9] I. Etikan, S.A. Musa, R.S. Alkassim, Comparison of convenience sampling and purposive sampling, Am. J. Theor. Appl. Stat. 5 (1) (2016) 1–4, doi:10.11648/j.ajtas.20160501.11.

[10] E. Woode, A. Alhassan, C.S. Abaidoo, Effect of xylopic acid on sex hormones and spermatogenesis in male rats, Al. Ameen J. Med. Sci. 5 (3) (2012) 288–297.
[11] M.J.F. Luthfi, A simple and practical method for rat epididymal sperm count (*Rattus norvegicus*), Bio. Med. Nat. Prod. Chem. 4 (1) (2015) 1–3, doi:10.14421/biomedich.2015.41.1-3.
[12] S. La Vignera, R.A. Condorelli, G. Balercia, E. Vicari, A.E. Calogero, Does alcohol have any effect on male reproductive function? A review of literature, Asian J. Androl. 15 (2) (2013) 221–225, doi:10.1038/aja.2012.118.
[13] S. Awe, J. Makinde, Antimalarial effects of the stem bark aqueous extracts of three Kaya species, Fitoterapia 62 (6) (1991) 467–473.
[14] D.D. Obiri, N. Osafo, P.G. Ayande, A.O. Antwi, Xylopia aethiopica (Annonaceae) fruit extract suppresses Freund′ s adjuvant-induced arthritis in Sprague-Dawley rats, J. Ethnopharmacol. 152 (3) (2014) 522–531, doi:10.1016/j.jep.2014.01.035.
[15] E. Woode, E.O. Ameyaw, E. Boakye-Gyasi, W.K.M. Abotsi, J. Oppong Kyekyeku, R. Adosraku, et al., Effects of an ethanol extract and the diterpene, xylopic acid, of xylopia aethiopica fruits in murine models of musculoskeletal pain, Pharm. Biol. 54 (12) (2016) 2978–2986, doi:10.1080/13880209.2016.1199040.
[16] R.P. Biney, C.K. Benneh, E.O. Ameyaw, E. Boakye-Gyasi, E. Woode, Xylopia aethiopica fruit extract exhibits an antidepressant-like effect via interaction with serotonergic neurotransmission in mice, J. Ethnopharmacol. 184 (2016) 49–57, doi:10.1016/j.jep.2016.02.023.
[17] K. Annan, R. Dickson, Evaluation of wound healing actions of hoslundia opposita vahl, anthocleista nobilis G. don. and balanites aegyptiaca l, J. Sci. Technol. 28 (2) (2008) 26–35, doi:10.4314/just.v28i2.33091.
[18] M. Thomson, K. Al-Qattan, S. Al-Sawan, M. Alnaqeeb, I. Khan, M. Ali, The use of ginger (Zingiber officinale rosc.) as a potential anti-inflammatory and antithrombotic agent, Prostag. Leukotr. Ess. 67 (6) (2002) 475–478, doi:10.1054/plef.2002.0441.
[19] J.A. Apenteng, M. Ogundeyi, E.E. Oppong, C. Osei-Asare, M.G. Brookman-Amissah, In vitro anti-infective and antioxidant activity of xylopia aethiopica [Dun.] A. rich: a comparison of the fruits and leaves extracts, J. Med. Plants 4 (5) (2016) 24–29.
[20] L. Marius, T.E. Rakiatou, O.E. Noufou, K. Felix, T. Andre, D. Pierre, G.I. Pierre, In vitro antioxidant activity and phenolic contents of different fractions of ethanolic extract from *Khaya senegalensis* A. Juss. (Meliaceae) stem barks, Afri. J Pharm. Pharmacol. 10 (23) (2016) 501–507, doi:10.5897/ajpp.2016.4564.
[21] K.G. Ngwoke, I.A. Anusi, P.M. Eze, U.M. Okezie, C.C. Abba, D.O. Abonyi, Phytochemical and antioxidant properties of stem bark extracts of anthocleista nobilis, Eur. J. Med. Plants 8 (2) (2015) 107–111, doi:10.9734/ejmp/2015/16107.
[22] A. William, U.K. Sandabe, V.A. Mania, B. Paul-Bokko, B.U. Shamaki, I.M. Wiam, F.I. Abdulrahman, P.A. Onyeyili, Effects of the ethanolic stem bark extract of khaya senegalensis on some sperm parameters in male albino rats, Continental J. Biol. Sci. 9 (1) (2016) 25–35.
[23] E.K. Nwangwa, Antifertility effects of ethanolic extract of *Xylopia aethiopica* on male reproductive organ of Wistar rats, Am. J. Med. Sci. 2 (1) (2012) 12–15, doi:10.5923/j.ajmms.20120201.03.
[24] D. Creasy, A. Bube, E.D. Rijik, H. Kandori, M. Kuwahara, R. Masson, P. Rogerson, Proliferative and non-proliferative lesions of the rat and mouse male reproductive system, Toxicol. Path 40 (6_suppl) (2012) 40S–121S, doi:10.1177/0192623312454337.
[25] S. Aboulmaouahib, A. Madkour, I. Kaarouch, O. Sefrioui, B. Saadani, H. Copin, …, R. Cadi, Impact of alcohol and cigarette smoking consumption in male fertility potential: looks at lipid peroxidation, enzymatic antioxidant activities and sperm DNA damage, Andrologia 50 (3) (2018) e12926, doi:10.1111/and.12926.
[26] A.A. Oremosu, E.N. Akang, Impact of alcohol on male reproductive hormones, oxidative stress and semen parameters in Sprague–Dawley rats, Middle East Fertil. Soc. J. 20 (2) (2015) 114–118, doi:10.1016/j.mefs.2014.07.001.